CLAIMS

1. A method for producing a heterologous RNA of interest, which method is characterized in that it comprises at least the following steps:

(1) transforming the mitochondria of yeast cells lacking mitochondrial DNA with a mitochondrial transcription vector comprising at least one copy of the DNA encoding said heterologous RNA of interest under the control of regulatory element(s) for mitochondrial transcription, and a mitochondrial transformation reporter gene or a fragment of said reporter gene;

(2) identifying the yeast mitochondrial transformants that have incorporated the DNA of interest;

(3) culturing the yeast mitochondrial transformants selected in step (2);

(4) isolating the mitochondria from the yeast mitochondrial transformants obtained in step (3), and

(5) extracting and purifying the heterologous RNA of interest from said mitochondria.

2. The method as claimed in claim 1, characterized in that said yeast cells lacking mitochondrial DNA are *rho*^{0} cells.

3. The method as claimed in claim 1 or claim 2, characterized in that said cells lacking mitochondrial DNA are obtained from a Δ*SUV3* or Δ*DSS1* strain.

4. The method as claimed in any one of claims 1 to 3, characterized in that said cells lacking mitochondrial DNA comprise a chromosomal copy of a gene encoding an exogenous RNA polymerase and including a mitochondrial targeting signal.

5. The method as claimed in any one of claims 1 to 4, characterized in that said DNA encoding the RNA of interest is under the control of a promoter and a transcription terminator that are functional in

yeast mitochondria.

6. The method as claimed in any one of claims 1 to 5, characterized in that said mitochondrial transformation reporter gene is a gene encoding one of the proteins of a yeast respiratory chain.

7. The method as claimed in any one of claims 1 to 6, characterized in that said mitochondrial transcription vector comprises the sequence of an origin of replication of the mitochondrial DNA.

8. The method as claimed in any one of claims 1 to 7, characterized in that the transformation according to step (1) comprises the adsorption of said mitochondrial transcription vector onto metal microprojectiles and the projection of said microprojectiles onto said cells.

9. The method as claimed in any one of claims 1 to 8, characterized in that step (1) comprises the cotransformation of said yeast cells with said mitochondrial transcription vector and a vector that is replicative in yeast, comprising a nuclear selection marker.

10. The method as claimed in claim 9, characterized in that said nuclear marker is an auxotrophic marker of said transformed cells.

11. The method as claimed in any one of claims 1 to 10, characterized in that step (2) comprises:

(a_0) crossing the yeast mitochondrial transformants obtained in step (1) with a yeast tester strain of *rho*$^+$ mit$^-$ type,

(b_0) identifying the mitochondrial transformants which, once crossed, give diploid cells capable of growing on a non-fermentable medium, and

(c_0) repeating said crossing until isolated yeast colonies identified as being mitochondrial transformants carrying the mitochondrial transformation vector are obtained.

12. The method as claimed in claim 9 or claim 10, characterized in that step (2) comprises:

(a_1) a first selection or preselection of the yeast cells by means of said nuclear marker, by culturing in an appropriate medium,

(b_1) a second selection from the yeast cells selected in (a_1), in accordance with steps (a_0), (b_0) and (c_0), as defined in claim 11.

13. The method as claimed in any one of claims 1 to 12, characterized in that the isolation of the mitochondria, in accordance with step (4) of the method, comprises lysis or grinding of said cells, and then at least two centrifugation steps, at speeds preferably of between 750 g and 12 500 g, and recovery of the final centrifugation pellet.

14. The method as claimed in any one of claims 1 to 13, characterized in that step (5) advantageously comprises:

- eliminating the contaminating nucleic acids in the presence of appropriate buffers, the first buffer comprising at least one divalent ion-chelating agent, and the second buffer comprising an RNase and, optionally, a DNase,
- lysing the mitochondria in the presence of at least one detergent and a divalent ion-chelating agent and within a pH range of between 7 and 8, and
- isolating and purifying the RNA of interest.

15. A modified yeast cell, characterized in that it lacks mitochondrial DNA and in that it comprises a chromosomal copy of a gene encoding an exogenous RNA polymerase and including a mitochondrial targeting signal.

16. A modified yeast cell, characterized in that it lacks mitochondrial DNA and in that its mitochondria are transformed with a mitochondrial transcription vector as defined in claims 1 and 5 to 7.

17. The modified yeast cell as claimed in claim 15 or claim 16, characterized in that it is obtained from a Δ*SUV3* or Δ*DSS1* strain.

18. The modified yeast cell as claimed in

any one of claims 15 to 17, characterized in that it is obtained from a *rho*^{0} strain.

19. The use of yeast mitochondrial transformants lacking mitochondrial DNA, for the industrial production of a heterologous RNA of interest.

20. A system for carrying out the industrial production of a heterologous RNA of interest, characterized in that it comprises:

- yeast cells lacking mitochondrial DNA, in particular of *rho*^{0} strain, transformed with at least one mitochondrial transcription vector as defined in claims 1 to 5 and 7,
- at least one suitable culture medium for selecting said transformed cells,
- yeast tester cells of *rho*$^+$ mit$^-$ type,
- appropriate fermenters and culture media, and
- appropriate reagents for isolating the mitochondria from synthetic *rho*$^-$ cells and extracting the heterologous RNA of interest therefrom.